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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/54, 9/12, 15/10, 1/21, C12Q 1/68	A2	(11) International Publication Number: WO 95/14782 (43) International Publication Date: 1 June 1995 (01.06.95)

(21) International Application Number: PCT/US94/13554

(22) International Filing Date: 22 November 1994 (22.11.94)

(30) Priority Data:
08/156,020 23 November 1993 (23.11.93) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/156,020 (CIP)
Filed on 23 November 1993 (23.11.93)

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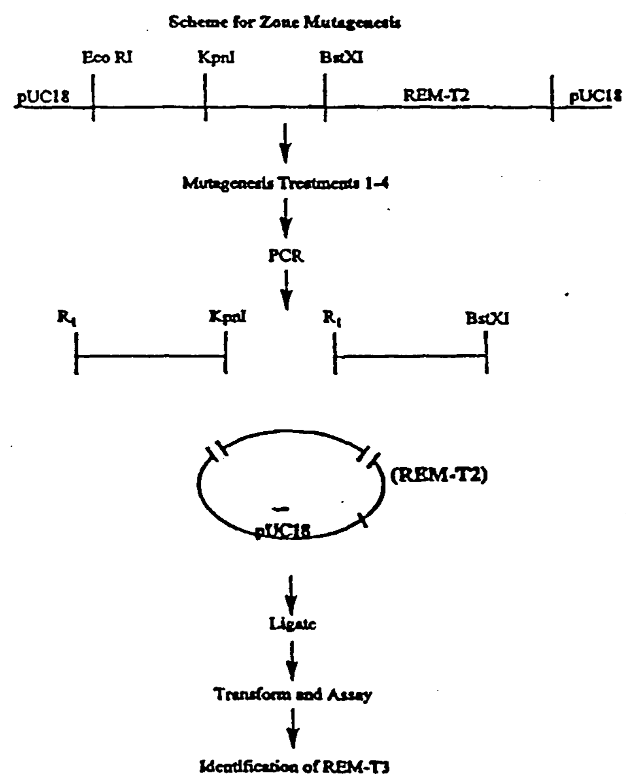
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: A MODIFIED THERMO RESISTANT DNA POLYMERASE

(57) Abstract

Novel, modified *Taq* DNA polymerases and genes encoding for them are disclosed. The modified *Taq* DNA polymerases of the invention are the same size, have the same heat stability and synthesis rate as the native enzyme, but lack the 5'-3' exonuclease activity. As a result of this modification, the enzymes have improved processivity as compared to the native enzyme. The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing, and DNA synthesis.



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MODIFIED THERMO-RESISTANT DNA POLYMERASES

BACKGROUND OF THE INVENTION

"This invention was made with Government support under grant GM 24711 awarded by the National Institutes of Health. The Government has certain rights in the invention."

Field of the Invention

This invention relates to the field of DNA polymerases for use in the polymerase chain reaction and DNA sequencing.

Description of the Prior Art

Polymerase Chain Reaction (PCR) was one of the most important inventions developed in area of biotechnology during the 1980's and has proven useful for a variety of tasks. *PCR Technology, Principles and Applications for DNA Amplification* (Erlich ed. 1989). The process provides a method for amplifying known specific nucleic acid sequence. Mullis, U.S. Pat. No. 4,683,202. The process comprises treating single or double stranded DNA containing the sequence of interest with an excess of two oligonucleotide primers sufficiently complementary of the strands so as to hybridize to the denatured strands. The hybridized primers are then extended by a DNA polymerase in the presence of the four dNTPs. The primer extension products are then separated and can serve as templates for another cycle of replication. The number of DNA templates approximately doubles on each cycle of amplification. Thus, 20 cycles of the process will result in approximately a 2^{20}-fold amplification.

The original protocols for PCR used the Klenow fragment of *E. coli* DNA polymerase I to catalyze the extension of the oligonucleotide primers. Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.* 51, 263 (1986); Mullis and Faloona, *Methods*

Enzymol. **155**, 335 (1987). The Klenow fragment proved somewhat cumbersome to use. Denaturation of the double stranded DNA at the start of each cycle requires temperatures ranging from 80 to 105°C. These temperatures inactivate the Klenow fragment. Consequently, fresh enzyme was required at the start of each new amplification cycle. While this process generally worked well for small segments of DNA (< 200 bp), a host of problems arose when replication of larger fragments was attempted.

The difficulties associated with use of the Klenow fragment DNA polymerase were circumvented with the introduction of thermostable DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus* (*Taq* DNA polymerase). Saiki et al., *Science* **239**, 487 (1989); Gelfand et al., U.S. Pat. No. 4,889,818. This enzyme has been cloned, overproduced, and the DNA sequence determined. Lawyer et al., *J. Biol. Chem.* **264**, 6427-6437 (1989).

In addition to its DNA polymerase activity, *Taq* DNA polymerase also possesses 5′-3′ polymerization-dependent exonuclease activity, but it lacks 3′-5′ exonuclease activity. Longley et al., *Nuc. Acids Res.* **18**, 7317-7322 (1990); Blanco et al., *Gene* **100**, 27-38 (1991); Bernad et al., *Cell* **59**, 219-228 (1989); Lawyer et al., *supra*; Holland et al., *Proc. Natl Acad. Sci.* **88**, 7276-7280 (1991); and Kelly and Joyce, *J. Mol. Biol.* **164**, 529-560 (1983). Studies have identified the 5′-3′ exonuclease activity as being an intrinsic part of *Taq* DNA polymerase. Longely et al., *supra*; and Barnes et al., *Gene* **112**, 29-35 (1992). This activity appears to facilitate a nick translation DNA reaction.

Native *Taq* DNA polymerase suffers from a high rate of misincorporation — about four times higher than that of the Klenow fragment of *E. coli* DNA polymerase I.

It has been estimated that *Taq* DNA polymerase incorporates one incorrect nucleotide in 9000. Tindall and Kunkel, *Biochemistry* 27, 6008 (1988). After 20 amplification cycles, this would result in DNA molecules with random mutations averaging one in every 900 bases. Saiki et al., *supra*. If the PCR product is to be inserted into an expression vector, the chance that one cloned molecule will contain an unwanted sequence alteration may be significant. It would be desirable, therefore, to decrease the rate of misincorporation of the DNA polymerase used in PCR without sacrificing the heat stability and rate of synthesis of the native *Taq* DNA polymerase.

It has been shown that removal of the 5′-235 codons of the *Taq* DNA polymerase gene results in an expression product that has no 5′-3′ exonuclease activity and a lower rate of mutagenesis. Tindall et al., *supra*; and Barnes, *supra*.

Other forms of *Taq* DNA polymerase are available. AmpliTaq™ is a commercially available genetically engineered version of *Taq* DNA polymerase and is substantially equivalent to the native form. Perkin Elmer Cetus; Saiki and Gelfand, *Amplifications* (Perkin Elmer Cetus), 1, 4 (1989). Also commercially available is a truncated gene product, the Stoffel fragment, that expresses an enzyme lacking the 5′-3′ exonuclease activity and having much lower unit activity, probably due to decreased processivity and increased mutagenesis. Barnes, *supra*. Gelfand and Abramson (PCT International Publication No. WO 92/06200) disclosed a modified *Taq* polymerase having the same length as the native enzyme, but with highly attenuated 5′-3′ exonuclease activity. The exonuclease activity is defeated by mutation in nucleotide 137 of the *Taq* polymerase gene, wherein the mutation is G

to A, resulting in a change in amino acid 46 of the enzyme from Gly to Asp. This enzyme is reported as having the same polymerase activity, processivity and extension rate as the native enzyme.

SUMMARY OF THE INVENTION

An object of this invention is to enhance the synthesis activity of DNA polymerase as used in PCR and DNA sequencing.

The invention disclosed herein achieves this object by providing a modified *Taq* DNA polymerase and a correspondingly modified *Taq* DNA polymerase gene sequence. The modified *Taq* DNA polymerase is the same size, has the same heat stability and synthesis rate as the native enzyme, but the 5′-3′ exonuclease activity is missing. As a result of this modification, the gene expression product has improved processivity.

The enzymes of the present invention enable improved methods of conducting PCR, DNA sequencing and DNA synthesis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical depiction of the restriction map of the *Taq* DNA polymerase gene.

Figure 2 is a graphical depiction of the method for producing the modified *Taq* DNA polymerase and the gene encoding it.

Figure 3 shows the sequencing primers for the pLSM5 (SEQ ID NO: 3) plasmid.

Figure 4 is a schematic depiction of the method for testing processivity used in trials 1 and 2.

Figure 5 is the autoradiograph showing the results of processivity testing used in trial 1.

Figure 6 is the autoradiograph showing the results of processivity testing used in trial 2.

Figure 7 is a schematic depiction of the method for testing processivity using PCR.

Figure 8 is the autoradiograph showing the results of processivity testing by the PCR method.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "replication product" refers to the oligonucleotides synthesized by DNA polymerase, whether it be as part of the polymerase chain reaction, DNA sequencing, or any other reaction where DNA polymerase is used to synthesize an oligonucleotide.

The term "oligonucleotide" as used herein is defined as a molecule composed of two or more deoxyribonucleotides or ribonucleotides.

The term "thermostable" refers to an enzyme that is stable to heat (> 95°C) and catalyzes combination of nucleotides to form an oligonucleotide. The term "thermo stability" as used herein refers to the characteristic stability of an enzyme to heat.

As used herein, the term "altered amino acid" means an amino acid that differs from that found in the native peptide or protein. Hence, if the native peptide

has the amino acid Cys at position 43, and the modified peptide has the amino acid Gly at that position, Gly is the "altered amino acid." Similarly, the term "altered nucleotide" means a nucleotide that differs from that found in a native oligonucleotide, polynucleotide, gene, or other nucleotide fragment.

As used herein, the phrase "lacking 5´-3´ exonuclease activity" means an enzyme having less than 1% of the 5´-3´ exonuclease activity of the native *Taq* DNA polymerase.

We undertook to inactivate the 5´-3´ exonuclease activity of the *Taq* DNA polymerase by *in vitro* mutagenesis without removal of the portion of the gene encoding that activity. The procedure followed was to develop a method of "zone mutagenesis" for that region of the *Taq* DNA polymerase gene encoding for the 5´-3´ exonuclease activity. *See* Figure 2. The nucleic acids encoding the amino acid residues required for 5´-3´ exonuclease activity have not been clearly identified, but earlier work suggests the region involved in DNA polymerases from other bacteria. Kelly and Joyce, *supra*.

To briefly summarize, using PCR technology we generated a *Taq* gene, which we cloned into the plasmid vector pUC18. *See* Figure 1. The pUC18 plasmid containing the *Taq* gene is designated pLSM5 (SEQ ID NO: 3). There are four base changes in the *Taq* gene produced by PCR and cloned in pLSM5 (SEQ ID NO: 3) compared to the published *Taq* DNA polymerase gene sequence (TTHTAQPIA in GenBank) (SEQ ID NO: 1): 1) C to G at position 89 in the untranslated 5´ end, 2) T to A at position 934 (Phe to Ile), 3) T to C at position 962 (Leu to Pro), and 4) G to A (no amino acid change) at position 2536. The protein expression product of this

gene has an altered amino acid at positions 272 (Ile) and 281 (Pro). We then subjected the pLSM5 (SEQ ID NO: 3) plasmid to conditions that would cause the random mutations in the 5´ exonuclease domain.

The vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) begins at nucleotide 70 and ends at 2619. The reading frame for translation begins at nucleotide 121 and ends at 2619 by the convention of Lawyer et al., *J. Biol. Chem.* **264**, 6427-6437 (1989).

The following sequence appears at the 5´ junction between the pUC18 plasmid and the *Taq* gene:

. . . AATTTCACACAGGAAACAGCTATGACCATGATTACG<u>AA</u>**TTCTAAA** . . . (SEQ ID NO: 14)

This sequence begins with the pUC18 antisense nucleotide sequence 490 to 455. The underlined nucleotides (<u>AA</u>) were added to create a restriction site. The *Taq* gene sequence (bold face) begins at nucleotide 70).

The following sequence appears at the 3´ junction between the pUC18 plasmid and the *Taq* gene:

....**CAAGGAGTGA**<u>GA</u>TTCTCTAGAGTCGACCTGCAGGCATGCAA GCTTGGCACTGGCCGTCGTTTT ... (SEQ ID NO: 15)

This sequence begins with *Taq* polymerase gene nucleotide 2610 to 2619. The underlined nucleotides (<u>GA</u>) were added to create a restriction site. The remaining sequence is the pUC18 antisense nucleotide, 413 to 381. Both junction sequences have been verified by sequence analysis.

The enzyme expression product of the pLSM5 plasmid, REM-T2 (SEQ ID NO: 4), has substantially the same processivity, 5´-3´ exonuclease activity, and

performance in normal PCR, to the extent tested so far, as the commercially available *Taq* DNA polymerase AmpliTaq™.

A variety of methods of mutagenesis are known to those of skill in the art and may be used in preparing a modified *Taq* DNA polymerase gene according to the present invention. *See, e.g.,* Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2d Ed. 1989). The mutated genes were then treated with restriction endonucleases that cut it in the region believed to be responsible for 5´-3´ exonuclease activity, thereby producing mutated inserts coding for that portion of the gene. A vector containing the native *Taq* DNA polymerase gene was treated with the same endonucleases and the previously obtained inserts ligated into the vector. Cells were transformed with the vector containing the inserts and colonies grown. We assayed polymerases expressed by the various colonies for polymerase activity as well as 5´-3´ exonuclease activity. The cells transfected with the gene encoding the modified *Taq* DNA polymerase meeting the objective of the present invention were thereby identified.

Appropriate host cells for the present invention may be chosen from the prokaryote group, which most frequently are represented by various strains of *E. coli*. Other microbial strains such as bacilli may be used, however. *Bacillus subtilis* and various species of *Pseudomonas* may be used, for example. In such prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species by Bolivar, et al., *Gene* **2**, 95 (1977). pBR322 contains genes for ampicillin and tetra-

cycline resistance, and thus provides addition markers that can be either retained or destroyed in constructing the desired vector. Commonly used prokaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequence, include such commonly used promoters as the β-lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., *Nature* **198**, 1056 (1977)), the tryptophan (trp) promoter system (Goeddel, et al., *Nucleic Acids Res.* **8**, 4057 (1980)), the lambda-derived P_L promoter (Shimatake et al., *Nature* **292**, 129 (1981)), and the N-gene ribosome binding site, which has been made useful as a portable control cassette (U.S. Pat. No. 4,711,845). The N-gene ribosome binding site comprises a first DNA sequence that is the P_L promoter operably linked to a second DNA sequence corresponding to N_{RBS} upstream of a third DNA sequence having at least one restriction site that permits cleavage within six bp 3′ of the N_{RBS} sequence. Also useful is the phosphatase A (phoA) system described by Chang et al. in European Patent Publication No. 196,864 published Oct. 8, 1986. Any available promoter system compatible with prokaryotes can be used, however.

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most used, although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated (Brach, *Meth. Enz.* **101**, 307 (1983)), other plasmid vectors suitable for yeast expression are known (*see, e.g.*, Stinchcomb et al., *Nature* **282**, 39 (1979), Tschempe et al., *Gene* **10**, 157 (1980), and Clarke et al., *Meth. Enz.* **101**, 300 (1983). Control sequences for yeast vectors include promoters

for the synthesis of glycolytic enzymes. Hess et al., *J. Adv. Enzyme Reg.* 7, 149 (1968) and Holland et al., *Biotechnology* 17, 4900 (1978).

Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255, 2073 (1980) and those for other glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other promoters that have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization. Holland, *supra.*

It is also believed that terminator sequences are desirable at the 3' end of the coding sequences. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland et al., *J. Biol. Chem.* 256, 1385 (1981) or the LEU2 gene obtained from YEp13 (Broach et al., *Gene* 8, 121 (1978). Any vector containing a yeast-compatible promoter, origin of replication, and other control sequence is suitable, however.

It is also possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. *See, e.g., Tissue Culture* (Cruz and Patterson eds., Academic Press 1973). Useful host cell lines include murine myelomas N51, VERO and HeLa cells, and Chinese Hamster Ovary (CHO) cells.

Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as the commonly used early and late promoters from Simian Virus 40 (SV 40) (Fiers et al., *Nature* 273, 113 (1978)) or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters. A system for expressing DNA in mammalian systems using the BPV as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. General aspects of mammalian cell host system transformations have been described by Axel, U.S. Pat. No. 4,399,216. It now appears that "enhancer" regions are important in optimizing expression. These generally are sequences found upstream of the promoter region. Origins of replication may be obtained from viral sources. Integration into the chromosome, however, is a common mechanism for DNA replication in eucaryotes.

Plant cells are also now available as hosts. Control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequence are available. Depicker et al., *J. Mol. Appl. Gen.* 1, 561 (1982).

In addition, expression systems employing insect cells utilizing the control systems provided by baculovirus vectors have been described. Miller et al, *Genetic Engineering* 8, 277-297 (Setlow et al. eds. Plenum Publishing 1986). These systems are also successful in producing *Taq* DNA polymerase.

Cells transformed with the modified *Taq* DNA polymerase gene may be grown using any suitable technique. The appropriate technique will depend on the cell type and will be known to those skilled in the art.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The treatment employing calcium chloride is used for prokaryotes or other cells that contain substantial cell wall barriers. Cohen, *Proc. Natl. Acad. Sci. (USA)* **69**, 2110 (1972). Infection with *Agrobacterium tumefaciens* is used for certain plant cells. Shaw et al. *Gene* **23**, 315 (1983). For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb is preferred. *Virology* **52**, 546 (1978). Transformations into yeast are carried out according to the method of Van solingen et al., *J. Bact.* **130**, 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA)* **76**, 3829 (1979).

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and relegated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. *See, e.g., New England Biolabs, Product Catalog.* In general, about 1 μg of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μl of buffer solution. Often excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid may be recovered from

aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

Cells producing enzyme of the desired type can be identified by standard techniques for assaying DNA polymerase and 5´-3´ exonuclease activity. *Id.* Using some of these methods, we were able to isolate a *Taq* DNA polymerase having the same size, heat stability, and synthetic activity of native *Taq* DNA polymerase, but having increased processivity and resulting in decreased mutagenesis of PCR DNA products. *See* examples *infra*.

The modified *Taq* DNA polymerase of the present invention was chosen from a colony producing the enzyme with a relatively high polymerase activity and low 5´-3´ exonuclease activity. We designated this product REM-T3 (SEQ ID NO: 6). An equivalent independently isolated product with a different mutation but equivalent properties is designated REM-T5 (SEQ ID NO: 8).

In addition to the modifications of native *Taq* DNA polymerase present in the modified *Taq* DNA polymerase of the present invention, individual amino acid residues in the peptide chain comprising the *Taq* DNA polymerase may be modified or deleted without eliminating any of the requisite properties described herein. Such alterations that do not destroy activity do not remove the DNA sequence or the modified *Taq* DNA polymerase from the contemplated scope of the present invention.

In order to assay the modified *Taq* DNA polymerase, REM-T3 (SEQ ID NO: 6), it was necessary to isolate it. We used the following novel, short isolation technique producing high purity enzyme quickly. Bacteria were grown overnight or to an OD at 600 nm of about 2.0 to 2.5 and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet washed with a solution of 50 mM Tris(8.0), 50 M dextrose, and 1 mM EDTA (15 x cell wt). The pellet was re suspended and lysed with a solution of 50 mM Tris, 50 mM dextrose, 1 mM EDTA, and 1 mg/ml lysozyme(5x cell wt). An equal volume of a solution of 10 mM Tris and 50 mM KCl, and 1 mM EDTA was added and the resulting mixture incubated at 75°C for 60 min before centrifuging at 8000 rpm for 15 min. The pellet was discarded and an equal volume of DEAE and 0.4 M KPO_4 (6.8) was added to the supernatant. The mixture was then incubated at 0°C for 30 min and then centrifuged at 10,000 rpm for 20 min. The pellet was discarded and the supernatant put on a phosphocellulose column with 0.02 M KPO_4 (7.5)(4x cell wt). The column was eluted with a gradient of 0.02 to 0.4 M KPO_4 (7.5). The peak was collected and applied to a Bio Rex-70 column with a solution of 0.02 M KPO_4 (7.6), 80 mM KCl 5%, glycerol, 0.5% Tween, and 0.5% Nonidet P-40. This column was then eluted with a step gradient of 0.3 M KCl and the peak collected.

The thermostability of the modified *Taq* DNA polymerase of the present invention must be substantially equivalent to that of native *Taq* DNA polymerase, i.e., it must not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids. The heating conditions (e.g., temperature and time) necessary

for denaturation will depend on a variety of factors, including the buffer salt concentration and the length and composition of the nucleotide chain. Typically, the temperature range for which the enzyme must be stable is about 90 to about 105°C for about 0.5 to four minutes. These values may vary depending on the conditions.

The modified *Taq* DNA polymerase of the present invention preferably functions optimally at temperatures above 40°C. The enzymes of the present invention is active in the temperature range 55 - 95°C, and preferably in the range 70 - 95°C.

U.S. Pat. No. 4,889,818 discloses and claims a native form of *Taq* DNA polymerase. Because the modified *Taq* DNA polymerase of the present invention retains all the characteristics of the native form that are useful in PCR technology, its use in PCR is preferable to the native form. Consequently, applications using *Taq* DNA polymerase as described in U.S. Pat. No. 4,889,818, col. 14, l. 33 to col. 27, l. 27 may also use the modified *Taq* DNA polymerase of the present invention. Accordingly, the disclosure of U.S. Pat. No. 4,889,818 is hereby incorporated by reference.

Besides use in the polymerase chain reaction, the modified *Taq* DNA polymerase of the present invention can be used in DNA sequencing by, for example, the Sanger dideoxy-mediated chain-termination method. Sanger et al., *Proc. Natl. Acad. Sci.* 74, 5463 (1977). Other similar uses will be known to those of skill in the art.

The following examples further elucidate the present invention, but are not intended to limit it.

EXAMPLE 1

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 1

The *Taq* polymerase gene was amplified from genomic DNA (*Thermus aquaticus*) using primers adding an EcoRI site in the 5´ UTR (nucleotide 70) and BglI site at the 3´ end (nucleotide 2619). The the PCR product was cloned into pUC18 after digesting the vector with EcoRI and BamHI. *See* Figure 1. We designated this *Taq* gene REM-T2. We then incubated the plasmid containing the *Taq* gene at pH 4.8 (10 mM sodium acetate) and room temperature for 20 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

The pLSM5 (SEQ ID NO: 3) vector was digested with EcoRI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5´-3´ exonuclease activity.

EXAMPLE 2

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 2

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We incubated the plasmid DNA containing the

Taq gene at pH 4.8 and 60°C for 5 minutes followed by neutralization to pH 8.0 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)) followed by digestion with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5´-3´ exonuclease activity.

EXAMPLE 3

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 3

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We amplified the N-terminal region of the *Taq* DNA polymerase gene for three consecutive PCR programs of 30 cycles each using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC) and the "sequencing primer" 628A (CCC AAA GCC AGG CCG (SEQ ID NO: 12)). Inserts for the putative amino terminal region of the gene were generated by digestion of the PCR products with Eco RI and KpnI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and KpnI and purified.

The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5´-3´ exonuclease activity.

EXAMPLE 4

Zone Mutagenesis of the *Taq* DNA Polymerase Gene - Treatment 4

Using PCR, we generated a *Taq* gene (REM-T2), which we cloned into the plasmid vector pUC18. *See* Figure 1. We incubated the plasmid DNA containing the *Taq* gene a pH 4.8 and 70°C for 15 minutes followed by neutralization to pH 8 with 50 mM Tris HCl. Inserts for the putative amino terminal region of the gene were generated by PCR using the "reverse primer" for pUC18 (CAG GAA ACA GCT ATG ACC (SEQ ID NO: 11)) and the "sequencing primer" 1155A (CAG GTC CCT GAG GGC (SEQ ID NO: 13)) and 5x concentration of dNTPs (0.75 mM) followed by digestion with Eco RI and BstXI.

A vector encoding the *Taq* gene (pLSM5 (SEQ ID NO: 3) producing the enzyme REM-T2 (SEQ ID NO: 4)) was digested with Eco RI and BstXI and purified. The inserts previously generated were then inserted into this vector. Ligation for insertion of the modified *Taq* gene was followed by transformation of DH5a cells followed by growth of individual colonies with assay for the DNA polymerase activity and the 5´-3´ exonuclease activity.

EXAMPLE 5

DNA Polymerase Activity Assay

Assay mixture:

reaction volume: 0.3 ml

25 mM Tris-HCl (pH = 8.8)

4 mM $MgCl_2$

22 μg activated ssDNA (salmon sperm)

0.033 mM dNTP (each)

2 μCi [methyl-^{3}H] thymidine 5′ triphosphate

enzyme

Assay procedure:

The mixture was incubated at 75°C for 10 minutes. The reaction was stopped with 2 ml ice cold 10% TCA - 0.1 M sodium pyrophosphate. The tubes were then placed on ice for 10 minutes and the reaction volume filtered. The tube and filter were washed three times with 2 ml of 10% TCA - 0.1 M sodium pyrophosphate. The filter was then washed with 10 ml 0.01 N HCl. Next the filters were dried at 120°C for 15 minutes. The dried filters were counted in 1 ml of Scintiverse.

The results are displayed in Table 1, *infra*.

EXAMPLE 6

5′-3′ Exonuclease Activity Assay

Preparation of double stranded substrate with blunt ends and removal of 5′ phosphate

A Blue-Script plasmid was cut with HincII to produce one double stranded piece with blunt ends and treated with CIP (calf intestine phosphatase) to remove the 5′ phosphate.

End-labeling of the 5′ ends using [g-\|o(.^{32})P]ATP

8 μl plasmid and 4 μl buffer were mixed with spermidine and 28 μl distilled H_2O. The mixture was then heated to 70°C for 5 minutes and then chilled on ice for 2 minutes. 10 μl kinase buffer with 1 μl [g-^{32}P]ATP (about 10 mCi) and 2 μl (20 units) of T4 polynucleotide kinase were added. Then the mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 2 μl 0.5 M EDTA. The enzyme was inactivated by incubating for 10 minutes at 70°C. The radioactive ATP was removed by washing 4 times (2 ml each) in Centricon 100. The final volume was about 50 μl (38,000 cpm/μl).

5′-3′ exonuclease assay

Assay conditions:

reaction volume 50 μl

25 mM Tris HCl (8.8)

4 mM Mg Cl_2

0.5 - 1 μl labeled substrate

0.3 units of DNA polymerase

Samples were incubated at 50 - 55°C for 15, 30 or 60 minutes. The reaction was stopped with 0.3 ml 10% TCA. The sample was microfuged for 15 minutes at 4°C. 0.1 ml was sampled on filter paper. The filter paper was dried at 120°C for 15 minutes. Dried filters were counted in 1 ml of Scintiverse.

The assay results are presented in Table 1, *infra*.

EXAMPLE 7

Sequencing Mutant Genes

Three mutants were chosen from those listed in Table 1 for low exonuclease activity. These were colony 18⁻ (the plasmid of which we designate pTarf2 (SEQ ID NO: 9)) and colony 20⁻ (the plasmid of which we designate pTarf3 (SEQ ID NO: 5)). A third mutant, pTarf5 (SEQ ID NO: 7), was obtained in a similar manner as in Example 4. pTarf3 (SEQ ID NO: 5) produces REM-T3 (SEQ ID NO: 6) and pTarf5 (SEQ ID NO: 7) produces REM-T5 (SEQ ID NO: 8). Bi-directional sequencing of the nucleic acid sequence of these mutants was conducted in the following manner: DNA sequence analysis was performed on alkaline-denatured double stranded plasmids. We used synthesized oligonucleotide primers (Fig. 3), [α-^{35}S]-dATP, and Sequenase® T7 DNA polymerase kit (United States Biochemical Corp.) according to the manufacturer's conditions. This method is based on the dideoxy chain termination reaction (Sanger, *Science* 214, 1205 (1981).

The alterations found in the mutants are presented in Table 2. These alterations are of the pLSM5 (SEQ ID NO: 3) sequence, i.e., the pTarf2 (SEQ ID NO: 9), pTarf3 (SEQ ID NO: 5), and pTarf5 (SEQ ID NO: 7) sequences are the same as the pLSM5 (SEQ ID NO: 3) sequence except for the alterations listed in Table 2.

TABLE 1

Enzyme Activity Of New *Taq* Clones

treatment	colony	polymerase act units/μl	5′-3′ exonuclease activity % of REM-T2 (SEQ ID NO: 4)
1	1	0.132	87
	2	0.503	97
	3	0.053	14
	4	0.27	88
	5	0.098	82
	6	0.41	94
	7	0.255	95
2	8	0.106	74
1	1′	1.54	104
	2′	1.60	94
	3′	1.06	105
	4′	1.49	100
	5′	1.06	104
	6′	2.20	114
	7′	0.35	107
	8′	0.68	117

	9	0.74	94
	10	0.87	109
2	11	1.81	98
	12	1.22	95
	13	1.68	110
	14	1.04	102
	15	0.84	101
	16	1.4	98
	17	0.15	104
	18	1.77	24
	19	1.11	107
3	20	1.73	0
	21	0.018	6
	22	0.48	0
	23	1.8	105
	24	0.83	94
	25	0.78	93

1 unit of polymerase activity = 10 nmoles of total nucleotides incorporated into acid insoluble form in 30 minutes at 75°C. Primed and unprimed colonies were obtained from cells transformed on different days.

TABLE 2

Alterations Relative to pLSM5 (SEQ ID NO: 3)

plasmid	nucleotide position	amino acid position	codon change	amino acid change
pTarf2 (SEQ ID NO: 9)	337	73	TTC-CTC	Phe-Leu
pTarf3 (SEQ ID NO: 5)	193 504	25 128	CGC-TGC AAG-AAA	Arg-Cys Lys-Lys
pTarf5 (SEQ ID NO: 7)	341	74	CGC-CAC	Arg-His

EXAMPLE 8

Improved Processivity of the Modified *Taq* Polymerase

Processivity of DNA synthesis by the modified Taq DNA polymerase (REM-T3) was assessed by several trials, with comparison to commercial enzymes and REM-T2. The method using the PCR protocol is novel.

Trial 1: Gel analysis of processivity by thermal stable DNA polymerases.

M13mp18 template (0.25 pmol/10 μl) and 5' ^{32}P-labeled 17-mer (M13/pUC-40, BioLabs) (0.50 pmol/10 μl) (calculated t_m = 52°C) were annealed in 40 μl of 10 mM Tris-HCl (pH 8.0), and 5 mM $MgCl_2$. The mixture was incubated for 3 minutes at 90°C, 20 minutes at 42°C, and 15 minutes at room temperature. The reaction mixture was adjusted to 200 μM each of dNTP, 0.05% Tween 20 and Nonidet P-40, 10 mM Tris-HCI (pH 8.0), 50 mM KCl and 2.5 mM $MgCl_2$, in a total

volume of 80 μl, then incubated at 55°C for 2 minutes without enzyme. Next, 0.94 units of enzyme (AmpliTaq™ (Cetus), Stoffel Fragment(Cetus), REM-T2 or REM-T3)/10 μl were added to start the reaction. Five μl aliquots were removed from the reaction mixture at 0, 15 , 30, 45 seconds, and 1, 2, and 5 minutes and added to 5 μl of stop solution (1 mg/ml each of xylene cyanol and bromphenol blue, 10 mM EDTA in formamide). For gel analysis, 5 μl were loaded onto a 6% wedge acrylamide/urea gel.

Figure 4 is a schematic depiction of the process and Figure 5 is an autoradiograph showing the results of trial 1.

<u>Trial 2: Gel analysis of processivity by thermal stable DNA polymerases.</u>

The same method was used as in Trial 1, except 0.22 units of polymerase/10 μl of reaction mixture were added. In addition, smaller volumes were used for annealing (25 μl) and reaction mixture (50 μl).

For trials 1 and 2, the assayed polymerase activity of the AmpliTaq™ was lower than usual. It appears from the gels that the number of actual units of AmpliTaq™ used in the reaction may have been higher that estimated and, therefore, may not be comparable to the other reactions.

Figure 6 shows the results of trial 2. Note that when the amount of polymerase is limiting, REM-T2 (SEQ ID NO: 4) and REM-T3 (SEQ ID NO: 6) have processivities greater than that of the Stoffel fragment.

Trial 3: PCR analysis of processitivity by thermal stable DNA polymerases.

The final volume of PCR reaction was 50 μl. The buffer contained 67 mM Tris-HCI (pH 8.8), 16 mM $(NH_4)_2SO_4$, 10 mM beta mercaptoethanol, 2 mM $MgCl_2$, 6.7 μM EDTA, and 150 μM each dNTP. There was an excess of template (0.02 pmol/10 μl) and primers (each 10 pmol/10 μl) over enzyme (0.04 units of polymerase/ 10 μl) for each PCR reaction. The template was pLSM5 (SEQ ID NO: 3), a 5.1 kb plasmid containing Taq DNA polymerase gene and used for sequencing. For the 834-951 primer set, at least 102 nucleotides must be added to the primers to form the 117 base pair product, and for the 1564 -1937 primer set, at least 358 nucleotides must be added to the primer to form the 373 base pair product . The PCR program was 20 sec denaturation at 94°C, 30 sec annealing at 48°C, and 2 min extension at 72°C for 12 cycles.

Figure 7 is a schematic depiction of this process and Figure 8 shows is an autoradiograph showing the results.

Interpretation of Processivity Testing

Trials I and 2 are based on methodology similar to Innis et al., *Proc. Natl. Acad. Sci.* 85, 9436 (1988); Tabor et al., *J. Biol. Chem.* 262, 16212 (1987); and Wernette et al., *Biochem.* 27, 6046 (1988). The use of a fixed primer for synthesis under conditions of limiting enzyme activity and excess template/primer allows analysis of the length of extension of the primer with minimal chance for re-initiation. Thus, analysis of product size by polyacrylamide/urea gel measures primer extension as a unit event, or processivity of the polymerase (trials 1 and 2).

Trial 3 is based on a new approach. We reasoned that it would be possible to measure processivity under conditions of PCR. With limiting enzyme concentration and excess primer/template concentration, the probability of re-initiation on a partially extended primer in PCR cycles is very low. Therefore, the length of the observed product (resulting from the complete extension of a primer through the opposing primer) is a measure of processivity. We found that 12 cycles results in sufficient yield to detect products with ethidium bromide on agarose gel. By varying the distance between primers we can determine a processivity range. AmpliTaq™, REM-T2, and REM-T3 have a processivity of at least 105 nucleotides, but less that 358 nucleotides. Stoffel Fragment, on the other hand has a processivity of less than 105 nucleotides.

Figure 8 compares the ability of four polymerases to extend a primer 105 nucleotides (Lanes 1-4) or 358 nucleotides (Lanes 5-8) under PCR conditions of excess DNA template (0.02 pmol/10 μl of reaction) and primer (10 pmol/10 μl of reaction) and limited polymerase units (0.04 units of polymerase/10 μl reaction). PCR products are shown on a 3% NuSieve gel. AmpliTaq™ is in lanes 1 and 5, Stoffel Fragment is in lanes 2 and 6, REM-T2 in lanes 3 and 7, and REM-T3 in lanes 4 and 8. Marker lane has ϕ x 174/Hae III.

It is evident from an examination of Figures 6, 7, and 8 that REM-T3 (SEQ ID NO: 6) has a processivity equal to or better than AmpliTaq™, and much better than the Stoffel fragment. This result demonstrates that the full length polypeptide of the modified *Taq* enzyme confers superior processivity compared to the truncated peptide of the Stoffel enzyme.

EXAMPLE 9

Misincorporation Rate for Modified *Taq* DNA Polymerases

Information already published by Barnes, *Gene* 112, 29-35 (1992) indicates that *Taq* DNA polymerase which has had the N-terminal region containing the 5' exonuclease domain removed has a diminished misincorporation rate. The information available indicates that such a modified *Taq* DNA polymerase has a two-fold lower misincorporation rate than native *Taq* DNA polymerase. Since the evidence presented by Barnes leads to the conclusion that the misincorporation by the *Taq* DNA polymerase is lowered in the absence of the exonuclease activity, we are motivated to measure the misincorporation rate of the modified *Taq* DNA polymerases described herein.

The assessment of misincorporation is done by several methodologies:

1. The methodology of Barnes uses a specially constructed plasmed with a flanking selectable marker, based on identification of *lacZ* as an indicator gene. Scoring for misincorporation in the *lac* gene is by the familiar blue/white test on an indicator dye (XGal). Testing for misincorporation is performed by inserting the plasmids into an indicator bacterial strain following PCR reactions *in vitro*.

2. The methodology of Tindall and Kunkel, *Biochemistry* 21, 6008-6013 (1988) monitors the fidelity of *in vitro* DNA synthesis using the *lacZ* gene for a complementation in a plasmid derived from M13 bacteriophage. Measurement of misincorporation is based on the blue/white test for *lacZ* function using an indicator dye in the plate. The plasmid derivative contains an open single-stranded gap region

of 390 nucleotides. This construction allows measurement of the forward mutation rate, or the substantially lower reversion mutation rate for any specific misincorporation constructed. The results found by Kunkel and coworkers, indicate that the native *Taq* DNA polymerase has a base substitution error rate of approximately 1/9000 nucleotides polymerized.

The processivity of our modified *Taq* DNA polymerase is much higher than the processivity of the truncated proteolytic fragment, and since the DNA polymerase literature indicates that misincorporation correlates with re-initiation, our misincorporation rate is considerably improved relative to native *Taq* DNA polymerase.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Moses M.D., Robb E.

(ii) TITLE OF INVENTION: Modified Thermo-Resistant DNA Polymerases

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 10 South Wacker Drive
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Greenfield Ph.D., Michael S.
(B) REGISTRATION NUMBER: 37,142
(C) REFERENCE/DOCKET NUMBER: 93,413

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (312)715-1000
(B) TELEFAX: (312)715-1234

(2) INFORMATION FOR SEQ ID NO::1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2626 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Thermus aquaticus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 121..2619

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 121..2616

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..2625
(D) OTHER INFORMATION: /note= "Native Taq DNA Polymerase nucleotide sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::1:

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GGGAGGCGTT TCTAAAAGCC CTTCAGGACG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC    120

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG      168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC      216
Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
             20                  25                  30

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC      264
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
         35                  40                  45

AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG      312
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
     50                  55                  60

GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG      360
Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
 65                  70                  75                  80

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC      408
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                 85                  90                  95

GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG      456
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG      504
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC      552
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG      600
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC      648
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC      696
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190
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CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG 744
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195 200 205

GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG 792
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210 215 220

AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG 840
Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225 230 235 240

CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG 888
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
245 250 255

GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC TTT 936
Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
260 265 270

CTG GAG AGG CTT GAG TTT GGC AGC CTC CTC CAC GAG TTC GGC CTT CTG 984
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275 280 285

GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG 1032
Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
290 295 300

GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT 1080
Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305 310 315 320

CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC 1128
Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
325 330 335

GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC 1176
Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
340 345 350

GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG 1224
Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
355 360 365

CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC 1272
Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
370 375 380

ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG 1320
Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385 390 395 400

GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG 1368
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
405 410 415

TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG 1416
Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG 1464
Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
435 440 445

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GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC     1512
Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC     1560
Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC     1608
Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC     1656
Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC     1704
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC     1752
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC     1800
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560

CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC     1848
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575

TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG     1896
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC     1944
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605

CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC     1992
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG     2040
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625                 630                 635                 640

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Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC     2136
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
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Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
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Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700
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GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG 2280
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GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG 2328
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
725 730 735

GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC 2376
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
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755 760 765

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Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770 775 780

GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC 2520
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785 790 795 800

CGG CTG GCC AAG GAG GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC 2568
Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
805 810 815

CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG 2616
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820 825 830

TGATACCACC 2626

(2) INFORMATION FOR SEQ ID NO::2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::2:

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20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80

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Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                 85                  90                  95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
            260                 265                 270

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
        275                 280                 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415
```

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
645 650 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
675 680 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
690 695 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705 710 715 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
725 730 735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
740 745 750

```
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830
```

(2) INFORMATION FOR SEQ ID NO::3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2626 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Thermus aquaticus

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(89, "g")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Taq DNA polymerase nucleotide sequence of C to G."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(934, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(962, "c")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(2535, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

```
(ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 121..2619

(ix) FEATURE:
     (A) NAME/KEY: mat_peptide
     (B) LOCATION: 121..2616

(ix) FEATURE:
     (A) NAME/KEY: -
     (B) LOCATION: 1..2619
     (D) OTHER INFORMATION: /note= "pLSM5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::3:

AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA      60

GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC     120

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG       168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC       216
Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
            20                  25                  30

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC       264
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
        35                  40                  45

AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG       312
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
    50                  55                  60

GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG       360
Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
 65                  70                  75                  80

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC       408
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                 85                  90                  95

GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG       456
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG       504
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC       552
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG       600
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC       648
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175
```

```
GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC     696
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG     744
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG     792
Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG     840
Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG     888
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT     936
Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG     984
Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG    1032
Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300

GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT    1080
Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC    1128
Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC    1176
Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350

GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG    1224
Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC    1272
Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG    1320
Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG    1368
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG    1416
Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430
```

```
GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG     1464
Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC     1512
Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC     1560
Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC     1608
Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC     1656
Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC     1704
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC     1752
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC     1800
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560

CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC     1848
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575

TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG     1896
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC     1944
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605

CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC     1992
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG     2040
Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                 630                 635                 640

GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC     2088
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC     2136
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG     2184
Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685
```

```
GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG      2232
Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG      2280
Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                 710                 715                 720

GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG      2328
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735

GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC      2376
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC      2424
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC      2472
Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC      2520
Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC      2568
Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG      2616
Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830

TGATACCACC                                                           2626
```

(2) INFORMATION FOR SEQ ID NO::4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::4:

```
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1               5                   10                  15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
            20                  25                  30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
        35                  40                  45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
    50                  55                  60
```

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
130 135 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
180 185 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
210 215 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
245 250 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
260 265 270

Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385 390 395 400

```
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                 630                 635                 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Gly Tyr Val
705                 710                 715                 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735
```

```
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830
```

(2) INFORMATION FOR SEQ ID NO::5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2626 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Thermus aquaticus

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(89, "g")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Taq DNA polymerase nucleotide sequence of C to G."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(934, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(962, "c")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(2535, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a

nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(193, "t")
(D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 193 of the native Taq DNA polymerase from C to T, resulting in an amino acid change of Arg to Cys."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(504, "a")
(D) OTHER INFORMATION: /note= "This mutation changes the nucleotide at position 504 of the native Taq DNA polymerase from G to A, which is conservative in nature."

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 121..2619

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 121..2616

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..2619
(D) OTHER INFORMATION: /note= "pTarf3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::5:

```
AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA      60

GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC     120

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG       168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

GTG GAC GGC CAC CAC CTG GCC TAC TGC ACC TTC CAC GCC CTG AAG GGC       216
Val Asp Gly His His Leu Ala Tyr Cys Thr Phe His Ala Leu Lys Gly
             20                  25                  30

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC       264
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
         35                  40                  45

AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG       312
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
     50                  55                  60

GTC TTT GAC GCC AAG GCC CCC TCC TTC CGC CAC GAG GCC TAC GGG GGG       360
Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
 65                  70                  75                  80

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC       408
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                 85                  90                  95
```

```
GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG      456
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAA      504
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC      552
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG      600
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC      648
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC      696
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG      744
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG      792
Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG      840
Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG      888
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT      936
Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG      984
Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG     1032
Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300

GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT     1080
Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC     1128
Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC     1176
Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350
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GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG      1224
Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC      1272
Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG      1320
Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG      1368
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG      1416
Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430

GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG      1464
Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC      1512
Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC      1560
Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC      1608
Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC      1656
Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC      1704
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC      1752
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC      1800
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560

CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC      1848
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575

TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG      1896
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC      1944
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605
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CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC     1992
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG     2040
Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                 630                 635                 640

GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC     2088
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC     2136
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG     2184
Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685

GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG     2232
Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG     2280
Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                 710                 715                 720

GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG     2328
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735

GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC     2376
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC     2424
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC     2472
Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC     2520
Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC     2568
Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG     2616
Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830

TGATACCACC                                                          2626
```

(2) INFORMATION FOR SEQ ID NO::6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 832 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::6:

```
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1               5                   10                  15

Val Asp Gly His His Leu Ala Tyr Cys Thr Phe His Ala Leu Lys Gly
            20                  25                  30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
        35                  40                  45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
    50                  55                  60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65                  70                  75                  80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                85                  90                  95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300
```

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625 630 635 640

```
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                 710                 715                 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830
```

(2) INFORMATION FOR SEQ ID NO::7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2626 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Thermus aquaticus

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(89, "g")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Taq DNA polymerase nucleotide sequence of C to G."

(ix) FEATURE:

(A) NAME/KEY: mutation
(B) LOCATION: replace(934, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(962, "c")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(2535, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(341, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 341 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation results in an amino acid change of Arg to His."

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 121..2619

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 121..2616

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..2619
(D) OTHER INFORMATION: /note= "pTarf5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::7:

```
AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA      60

GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC     120

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG       168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC       216
Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
             20                  25                  30

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC       264
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
         35                  40                  45
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AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG     312
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
     50                  55                  60

GTC TTT GAC GCC AAG GCC CCC TCC TTC CAC CAC GAG GCC TAC GGG GGG     360
Val Phe Asp Ala Lys Ala Pro Ser Phe His His Glu Ala Tyr Gly Gly
 65                  70                  75                  80

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC     408
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                 85                  90                  95

GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG     456
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG     504
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC     552
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG     600
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC     648
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC     696
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG     744
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG     792
Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG     840
Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG     888
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT     936
Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG     984
Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG    1032
Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300
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```
GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT      1080
Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC      1128
Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC      1176
Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350

GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG      1224
Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC      1272
Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG      1320
Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG      1368
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG      1416
Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430

GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG      1464
Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC      1512
Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC      1560
Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC      1608
Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC      1656
Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC      1704
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC      1752
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC      1800
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560
```

```
CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC    1848
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575

TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG    1896
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC    1944
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605

CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC    1992
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG    2040
Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                 630                 635                 640

GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC    2088
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC    2136
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG    2184
Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685

GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG    2232
Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG    2280
Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                 710                 715                 720

GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG    2328
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735

GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC    2376
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC    2424
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC    2472
Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC    2520
Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC    2568
Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815
```

```
CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG    2616
Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830

TGATACCACC                                                         2626
```

(2) INFORMATION FOR SEQ ID NO::8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::8:

```
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
            20                  25                  30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
        35                  40                  45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
    50                  55                  60

Val Phe Asp Ala Lys Ala Pro Ser Phe His His Glu Ala Tyr Gly Gly
 65                  70                  75                  80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                85                  90                  95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240
```

```
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                 535                 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                 550                 555                 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                 570                 575
```

```
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                 585                 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                 600                 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                 615                 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                 630                 635                 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                 650                 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                 665                 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                 680                 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                 695                 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                 710                 715                 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                 730                 735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                 745                 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                 760                 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                 775                 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830
```

(2) INFORMATION FOR SEQ ID NO::9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2626 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: mutation

(B) LOCATION: replace(89, "g")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at postion 89 of the native Taq DNA polymerase nucleotide sequence of C to G."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(934, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(962, "c")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(2535, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(337, "a")
(D) OTHER INFORMATION: /note= "This mutation results in a nucleotide alteration at position 337 of the native Taq DNA polymerase nucleotide sequence of T to C. This change results in an amino acid change of Phe to Leu."

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 121..2619

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 121..2616

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..2619
(D) OTHER INFORMATION: /note= "pTarf2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::9:

```
AAGCTCAGAT CTACCTGCCT GAGGGCGTCC GGTTCCAGCT GGCCCTTCCC GAGGGGGAGA      60

GGGAGGCGTT TCTAAAAGCC CTTCAGGAGG CTACCCGGGG GCGGGTGGTG GAAGGGTAAC     120

ATG AGG GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG      168
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
  1               5                  10                  15

GTG GAC GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC      216
```

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
20 25 30

CTC ACC ACC AGC CGG GGG GAG CCG GTG CAG GCG GTC TAC GGC TTC GCC 264
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35 40 45

AAG AGC CTC CTC AAG GCC CTC AAG GAG GAC GGG GAC GCG GTG ATC GTG 312
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50 55 60

GTC TTT GAC GCC AAG GCC CCC TCC CTC CGC CAC GAG GCC TAC GGG GGG 360
Val Phe Asp Ala Lys Ala Pro Ser Leu Arg His Glu Ala Tyr Gly Gly
65 70 75 80

TAC AAG GCG GGC CGG GCC CCC ACG CCG GAG GAC TTT CCC CGG CAA CTC 408
Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
85 90 95

GCC CTC ATC AAG GAG CTG GTG GAC CTC CTG GGG CTG GCG CGC CTC GAG 456
Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
100 105 110

GTC CCG GGC TAC GAG GCG GAC GAC GTC CTG GCC AGC CTG GCC AAG AAG 504
Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
115 120 125

GCG GAA AAG GAG GGC TAC GAG GTC CGC ATC CTC ACC GCC GAC AAA GAC 552
Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
130 135 140

CTT TAC CAG CTC CTT TCC GAC CGC ATC CAC GTC CTC CAC CCC GAG GGG 600
Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160

TAC CTC ATC ACC CCG GCC TGG CTT TGG GAA AAG TAC GGC CTG AGG CCC 648
Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
165 170 175

GAC CAG TGG GCC GAC TAC CGG GCC CTG ACC GGG GAC GAG TCC GAC AAC 696
Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
180 185 190

CTT CCC GGG GTC AAG GGC ATC GGG GAG AAG ACG GCG AGG AAG CTT CTG 744
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
195 200 205

GAG GAG TGG GGG AGC CTG GAA GCC CTC CTC AAG AAC CTG GAC CGG CTG 792
Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
210 215 220

AAG CCC GCC ATC CGG GAG AAG ATC CTG GCC CAC ATG GAC GAT CTG AAG 840
Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225 230 235 240

CTC TCC TGG GAC CTG GCC AAG GTG CGC ACC GAC CTG CCC CTG GAG GTG 888
Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
245 250 255

GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT AGG GCC ATT 936
Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
260 265 270

CTG GAG AGG CTT GAG TTT GGC AGC CCC CTC CAC GAG TTC GGC CTT CTG 984
Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
275 280 285

GAA AGC CCC AAG GCC CTG GAG GAG GCC CCC TGG CCC CCG CCG GAA GGG 1032
Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
290 295 300

GCC TTC GTG GGC TTT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC GAT 1080
Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305 310 315 320

CTT CTG GCC CTG GCC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC 1128
Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
325 330 335

GAG CCT TAT AAA GCC CTC AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC 1176
Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
340 345 350

GCC AAA GAC CTG AGC GTT CTG GCC CTG AGG GAA GGC CTT GGC CTC CCG 1224
Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
355 360 365

CCC GGC GAC GAC CCC ATG CTC CTC GCC TAC CTC CTG GAC CCT TCC AAC 1272
Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
370 375 380

ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC GGG GAG TGG ACG GAG 1320
Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385 390 395 400

GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC TTC GCC AAC CTG 1368
Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
405 410 415

TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG GAG 1416
Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

GTG GAG AGG CCC CTT TCC GCT GTC CTG GCC CAC ATG GAG GCC ACG GGG 1464
Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
435 440 445

GTG CGC CTG GAC GTG GCC TAT CTC AGG GCC TTG TCC CTG GAG GTG GCC 1512
Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
450 455 460

GAG GAG ATC GCC CGC CTC GAG GCC GAG GTC TTC CGC CTG GCC GGC CAC 1560
Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465 470 475 480

CCC TTC AAC CTC AAC TCC CGG GAC CAG CTG GAA AGG GTC CTC TTT GAC 1608
Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
485 490 495

GAG CTA GGG CTT CCC GCC ATC GGC AAG ACG GAG AAG ACC GGC AAG CGC 1656
Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
500 505 510

TCC ACC AGC GCC GCC GTC CTG GAG GCC CTC CGC GAG GCC CAC CCC ATC 1704
Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
515 520 525

GTG GAG AAG ATC CTG CAG TAC CGG GAG CTC ACC AAG CTG AAG AGC ACC 1752
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
530 535 540

TAC ATT GAC CCC TTG CCG GAC CTC ATC CAC CCC AGG ACG GGC CGC CTC 1800
Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545 550 555 560

CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG GGC AGG CTA AGT AGC 1848
His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
565 570 575

TCC GAT CCC AAC CTC CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG 1896
Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
580 585 590

AGG ATC CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC 1944
Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
595 600 605

CTG GAC TAT AGC CAG ATA GAG CTC AGG GTG CTG GCC CAC CTC TCC GGC 1992
Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610 615 620

GAC GAG AAC CTG ATC CGG GTC TTC CAG GAG GGG CGG GAC ATC CAC ACG 2040
Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625 630 635 640

GAG ACC GCC AGC TGG ATG TTC GGC GTC CCC CGG GAG GCC GTG GAC CCC 2088
Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
645 650 655

CTG ATG CGC CGG GCG GCC AAG ACC ATC AAC TTC GGG GTC CTC TAC GGC 2136
Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
660 665 670

ATG TCG GCC CAC CGC CTC TCC CAG GAG CTA GCC ATC CCT TAC GAG GAG 2184
Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
675 680 685

GCC CAG GCC TTC ATT GAG CGC TAC TTT CAG AGC TTC CCC AAG GTG CGG 2232
Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
690 695 700

GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG GGG TAC GTG 2280
Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705 710 715 720

GAG ACC CTC TTC GGC CGC CGC CGC TAC GTG CCA GAC CTA GAG GCC CGG 2328
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
725 730 735

GTG AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC 2376
Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
740 745 750

GTC CAG GGC ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC 2424
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
755 760 765

TTC CCC AGG CTG GAG GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC 2472
Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770 775 780

```
GAC GAG CTG GTC CTC GAG GCC CCA AAA GAG AGG GCG GAG GCC GTG GCC      2520
Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                 790                 795                 800

CGG CTG GCC AAG GAA GTC ATG GAG GGG GTG TAT CCC CTG GCC GTG CCC      2568
Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                 810                 815

CTG GAG GTG GAG GTG GGG ATA GGG GAG GAC TGG CTC TCC GCC AAG GAG      2616
Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                 825                 830

TGATACCACC                                                           2626
```

(2) INFORMATION FOR SEQ ID NO::10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::10:

```
Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1               5                   10                  15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
            20                  25                  30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
        35                  40                  45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
    50                  55                  60

Val Phe Asp Ala Lys Ala Pro Ser Leu Arg His Glu Ala Tyr Gly Gly
65                  70                  75                  80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
                85                  90                  95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
            100                 105                 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
        115                 120                 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
    130                 135                 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145                 150                 155                 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
                165                 170                 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
            180                 185                 190
```

```
Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
        195                 200                 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
    210                 215                 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
225                 230                 235                 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
                245                 250                 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Ile
            260                 265                 270

Leu Glu Arg Leu Glu Phe Gly Ser Pro Leu His Glu Phe Gly Leu Leu
        275                 280                 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
    290                 295                 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
305                 310                 315                 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
                325                 330                 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
            340                 345                 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
        355                 360                 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
    370                 375                 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
385                 390                 395                 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
                405                 410                 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
            420                 425                 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
        435                 440                 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
    450                 455                 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
465                 470                 475                 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
                485                 490                 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
            500                 505                 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
        515                 520                 525
```

```
Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
    530                     535                     540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                     550                     555                     560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                565                     570                     575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
            580                     585                     590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
        595                     600                     605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
    610                     615                     620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                     630                     635                     640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                645                     650                     655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
            660                     665                     670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
        675                     680                     685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
    690                     695                     700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                     710                     715                     720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
                725                     730                     735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
            740                     745                     750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
        755                     760                     765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
    770                     775                     780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                     790                     795                     800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
                805                     810                     815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            820                     825                     830
```

(2) INFORMATION FOR SEQ ID NO::11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "PCR reverse primer used for PUC18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::11:

CAGGAAACAG CTATGACC 18

(2) INFORMATION FOR SEQ ID NO::12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 11..15
(D) OTHER INFORMATION: /note= "PCR sequencing primer 628A used for pUC18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::12:

CCCAAAGCCA GGCCG 15

(2) INFORMATION FOR SEQ ID NO::13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "Sequencing primer 1155A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::13:

CAGGTCCCTG AGGGC 15

(2) INFORMATION FOR SEQ ID NO::14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..46
(D) OTHER INFORMATION: /note= "pUC18 - pLSM5 5' junction"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::14:

AATTTCACAC AGGAAACAGC TATGACCATG ATTACGAATT CTAAAA 46

(2) INFORMATION FOR SEQ ID NO::15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /note= "pUC18 - pLSM5 3' sequence junction"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO::15:

CAAGGAGTGA GATTCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCAC TGGCCGTCGT 60

TTT 63

What is claimed is:

1. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 934, 962, and 2535.

2. A modified *Taq* DNA polymerase gene according to claim 1 wherein the altered nucleotide at position 89 is G, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.

3. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 1.

4. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 2.

5. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 272 and 281.

6. A modified *Taq* DNA polymerase according to claim 5 wherein the altered amino acid at position 272 is Ile and the altered amino acid at position 281 is Pro.

7. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 193 and 504.

8. A modified *Taq* polymerase gene according to claim 7 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

9. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 193, 504, 934, 962, and 2535.

10. A modified *Taq* polymerase gene according to claim 9 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 193 is T, the altered amino acid at position 504 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A.

11. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 7.

12. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 8.

13. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 9.

14. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 10.

15. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 25.

16. A modified *Taq* DNA polymerase according to claim 15 wherein the altered amino acid at position 25 is Cys.

17. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 25, 272, and 281

18. A modified *Taq* DNA polymerase according to claim 17 wherein the altered amino acid at position 25 is Cys, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.

19. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at position 341.

20. A modified *Taq* polymerase gene according to claim 19 wherein the altered nucleotide at position 193 is T and the altered nucleotide at position 504 is A.

21. A modified *Taq* DNA polymerase gene essentially comprising the native *Taq* DNA polymerase gene having an altered nucleotide at positions 89, 341, 934, 962, and 2535.

22. A modified *Taq* polymerase gene according to claim 21 wherein the altered nucleotide at position 89 is G, the altered amino acid at position 341 is A, the altered nucleotide at position 934 is A, the altered nucleotide at position 962 is C, and the altered amino acid at position 2525 is A

23. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 19.

24. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 20.

25. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 21.

26. Host cells transfected with the modified *Taq* DNA polymerase gene of claim 22.

27. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at position 74.

28. A modified *Taq* DNA polymerase according to claim 27 wherein the altered amino acid at position 74 is His.

29. A modified *Taq* DNA polymerase essentially comprising the native *Taq* DNA polymerase with an altered amino acid at positions 74, 272, and 281

30. A modified *Taq* DNA polymerase according to claim 29 wherein the altered amino acid at position 74 is His, the altered amino acid at position 272 is Ile, and the altered amino acid at position 281 is Pro.

31. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

32. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

33. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

34. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

35. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

36. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.

37. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

38. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 38.

39. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

40. Polymerase chain reaction, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

41. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

42. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

43. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

44. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

45. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

46. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18.

47. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

48. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

49. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

50. DNA sequencing using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

51. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 5.

52. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 6.

53. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 15.

54. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 16.

55. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 17.

56. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 18

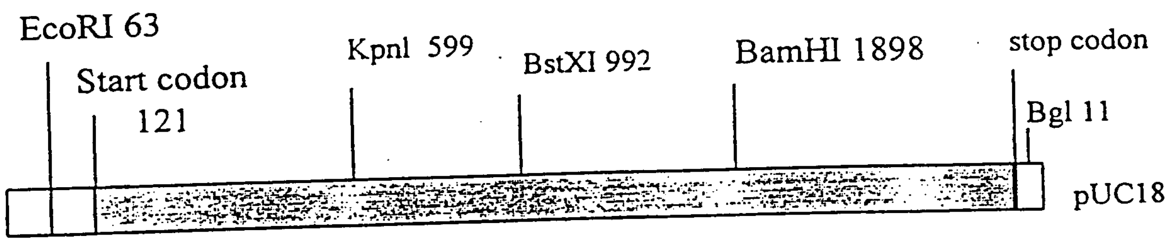
57. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 27.

58. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 28.

59. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 29.

60. DNA synthesis using a DNA polymerase, wherein the improvement comprises use of the modified *Taq* DNA polymerase of claim 30.

FIG. 1



Restriction map of gene for Taq DNA polymerase

FIG. 2

Scheme for Zone Mutagenesis

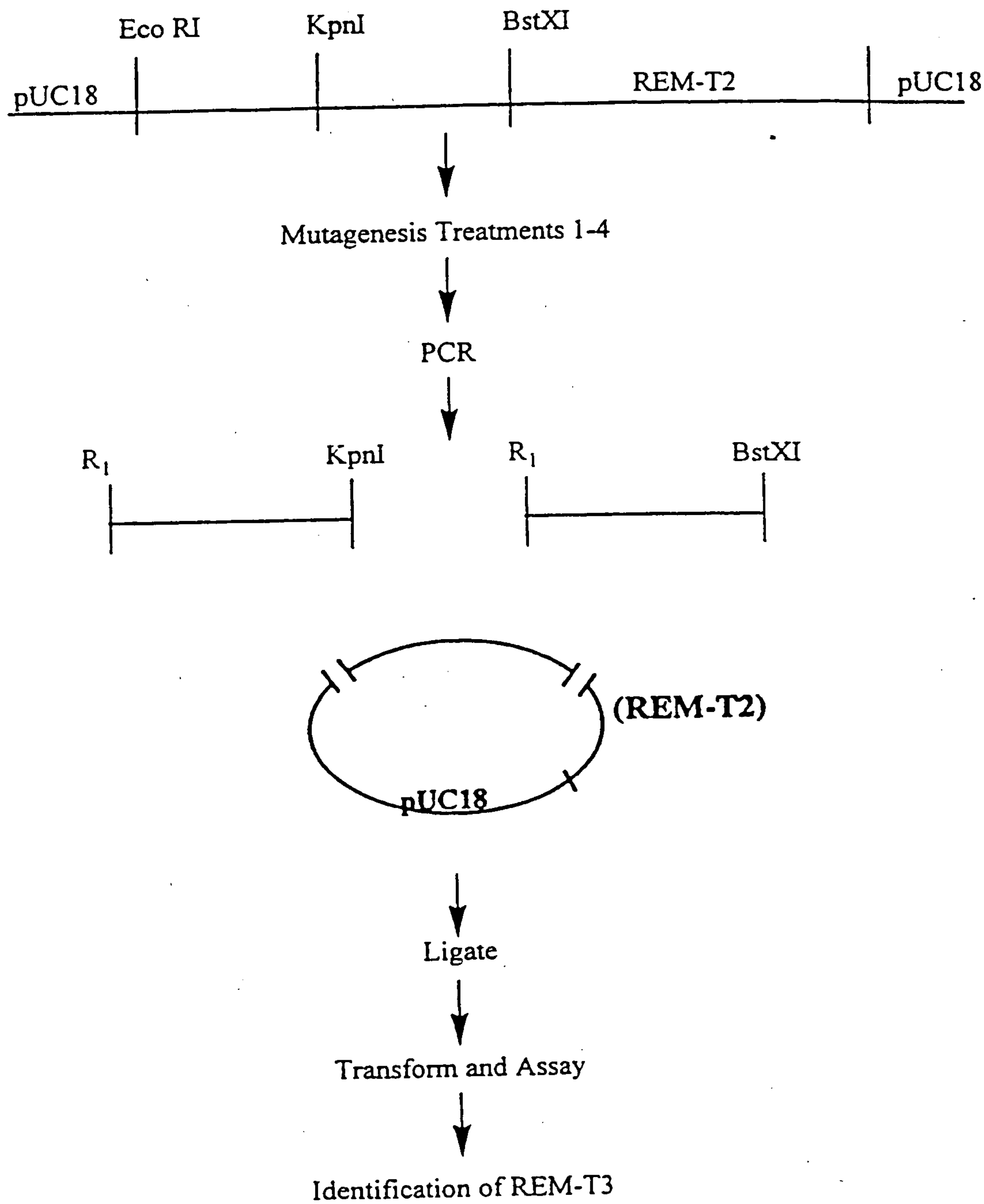


FIG. 3

Sequencing Primers for pLSM5

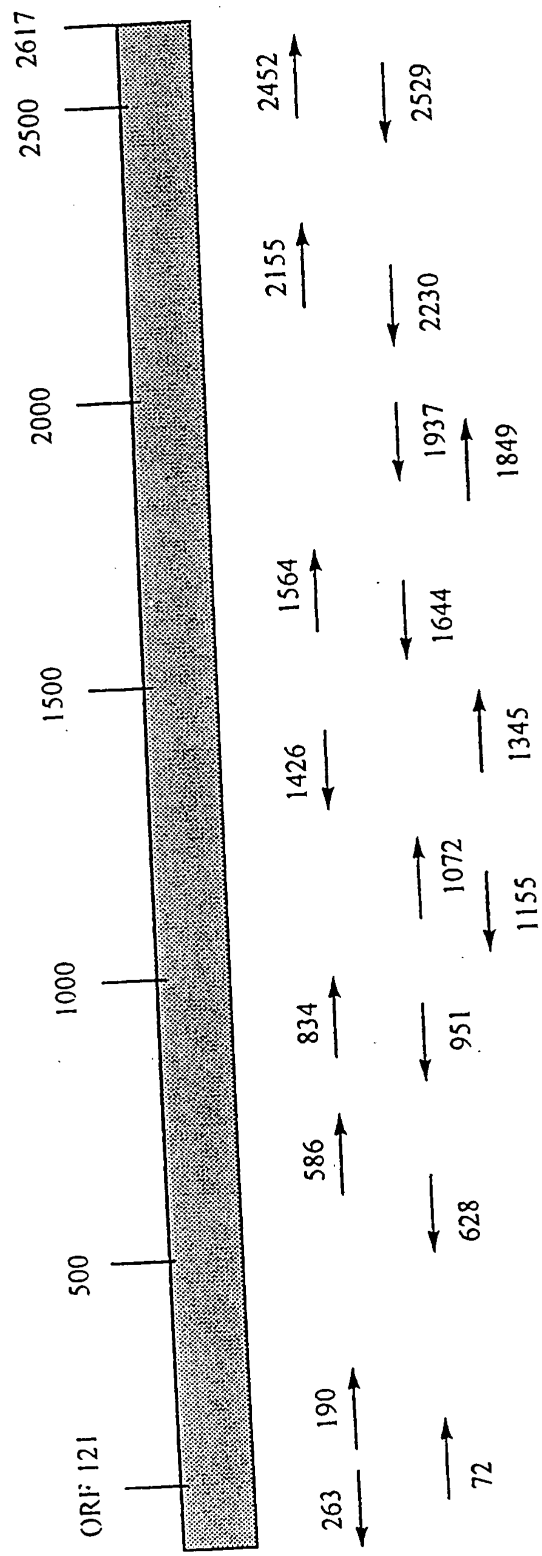
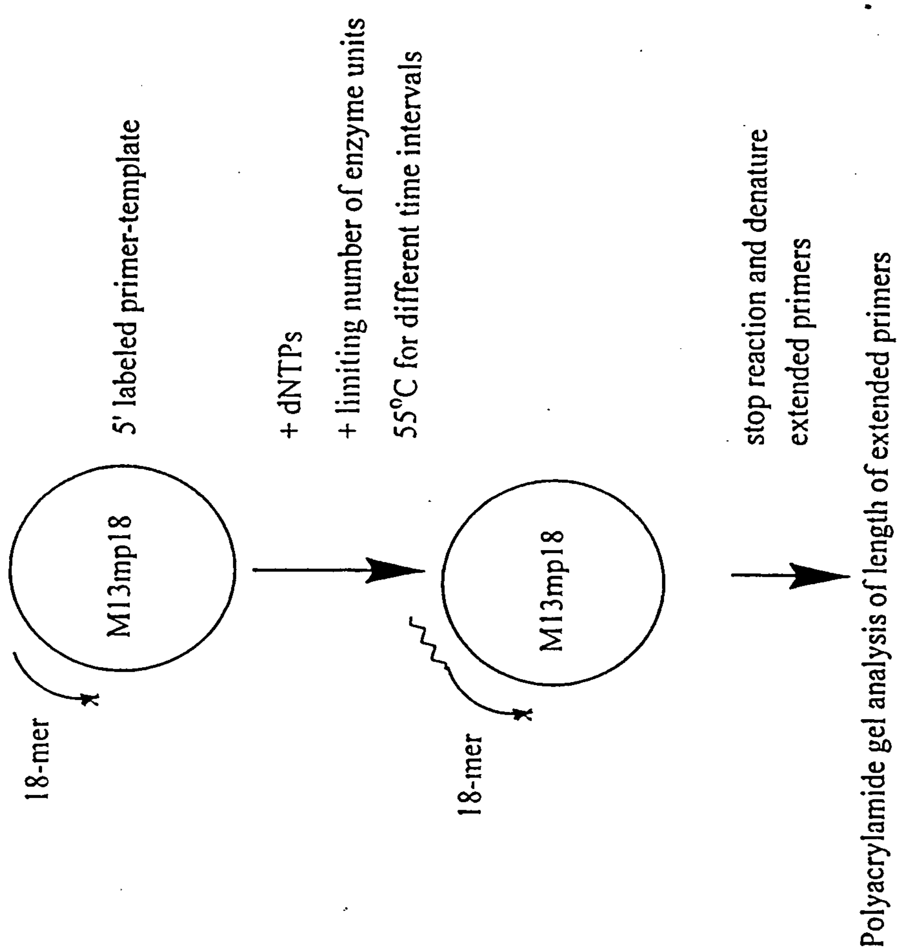


FIG. 4



18-mer
M13mp18
5' labeled primer-template
+ dNTPs
+ limiting number of enzyme units
55°C for different time intervals
18-mer
M13mp18
stop reaction and denature
extended primers
Polyacrylamide gel analysis of length of extended primers

FIG. 5

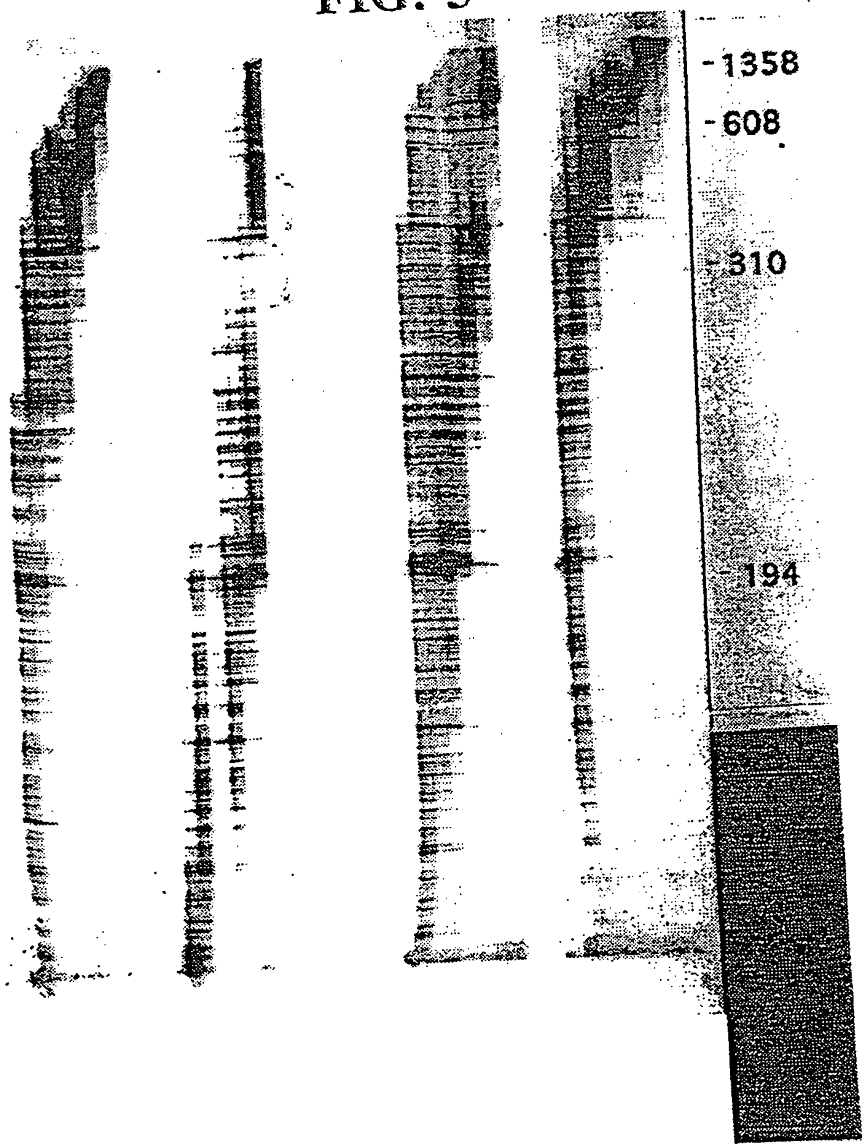


FIG. 6

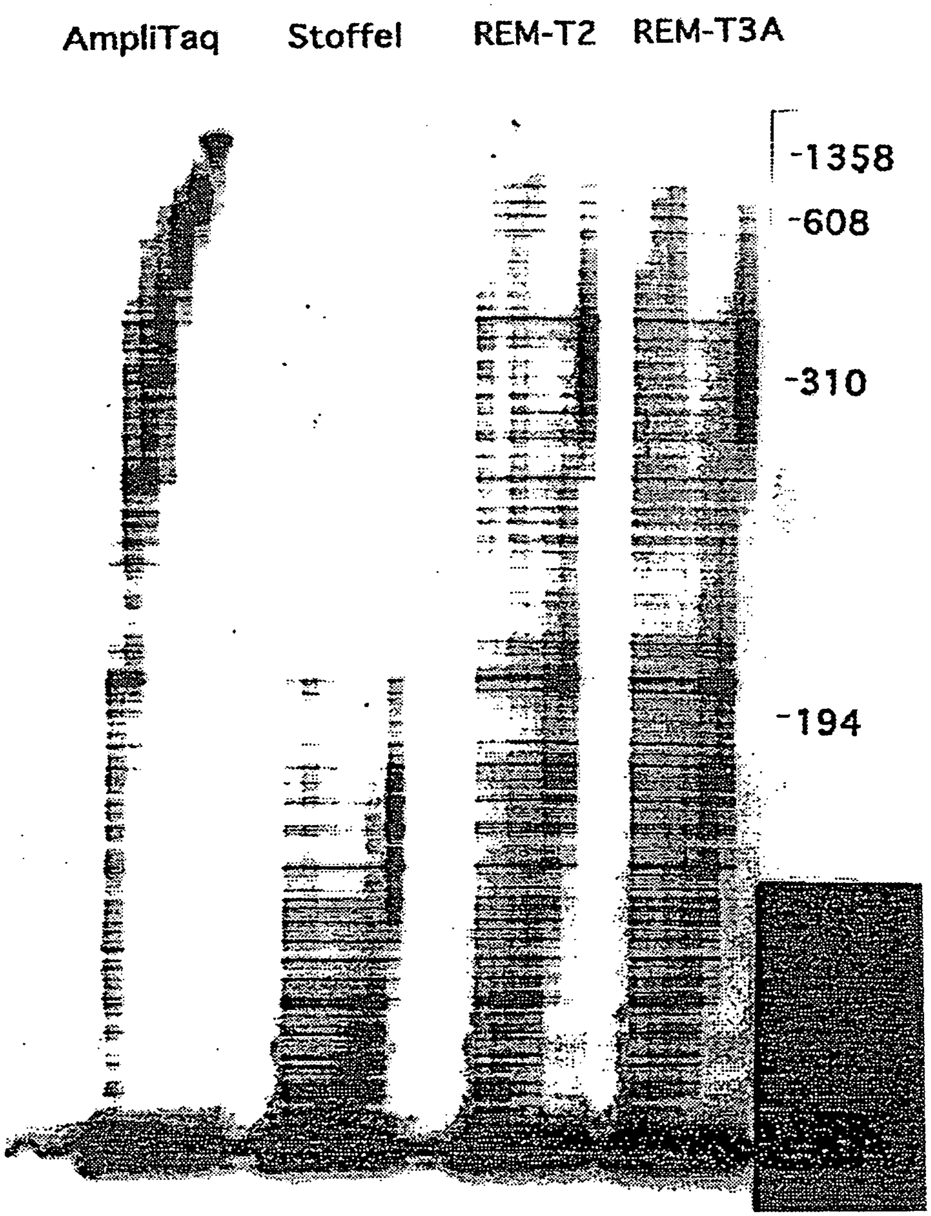


FIG. 7

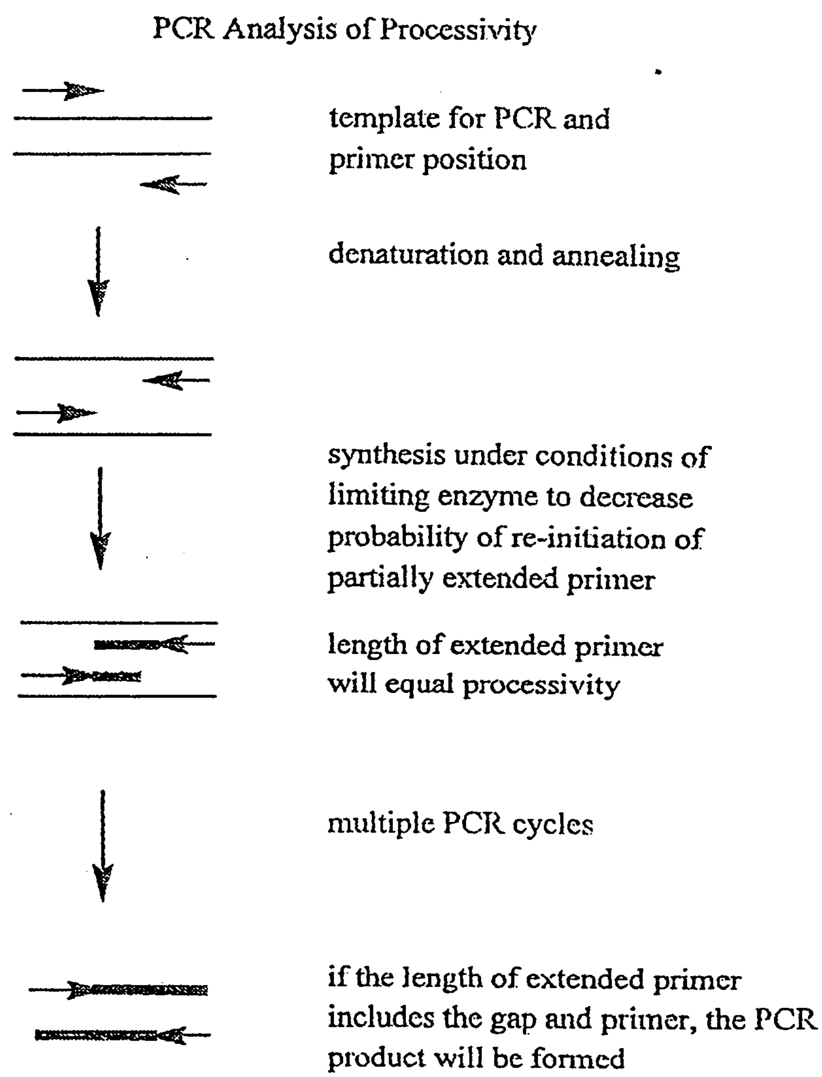

PCR Analysis of Processivity
template for PCR and primer position
denaturation and annealing
synthesis under conditions of limiting enzyme to decrease probability of re-initiation of partially extended primer
length of extended primer will equal processivity
multiple PCR cycles
if the length of extended primer includes the gap and primer, the PCR product will be formed

FIG. 8